Human TrkA (Luc) HEK293 Reporter Cell Data Sheet

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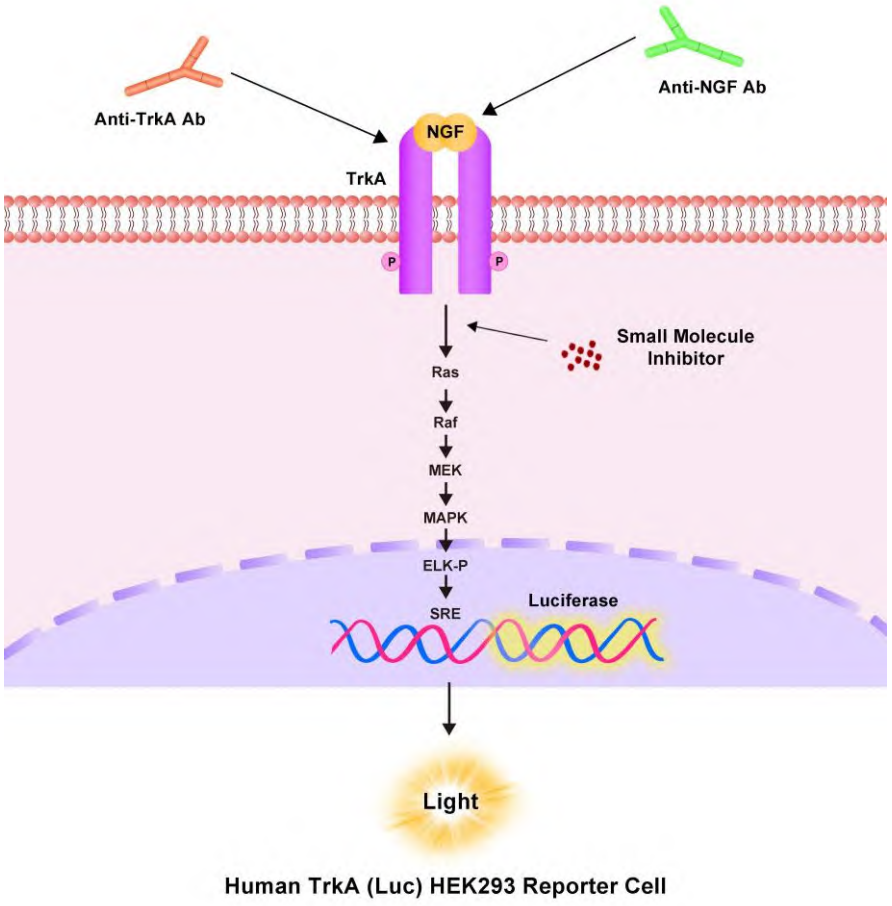
Catalog No.	Size
CHEK-ATF093	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TrkA (Luc) HEK293 Reporter Cell was engineered to not only express SRE signaling response element, but also express the receptor full length human TrkA (Uniprot： P04629-1). When stimulated with human NGF protein, the NGF/TrkA interaction drives SRE-mediated luminescence. Neutralization of biological effect of human NGF protein by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for neutralizing antibodies blocking the stimulation of human NGF protein.
- Screen for human TrkA small molecule inhibitor



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• *Cell Line Profile*

Cell line	Human TrkA (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin B (20 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

Note: If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ) in China, you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, 1%P/S
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin B (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37°C with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

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• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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- ***Receptor Assay***

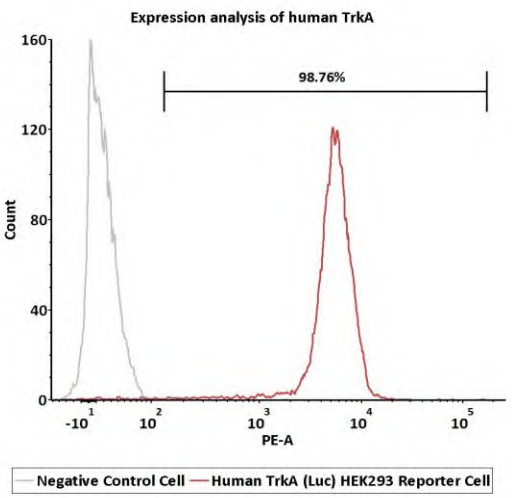

Fig1. Expression analysis of human TrkA on Human TrkA (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human TrkA (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human TrkA antibody.

- ***Signaling Bioassay***

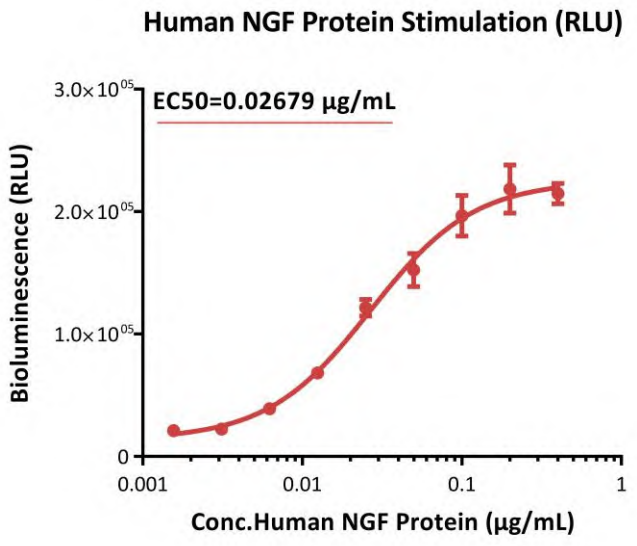

Fig2. Response to human NGF protein (RLU). The Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat. No. BEF-H5214). The EC50 was approximately 0.02679 μg/mL.

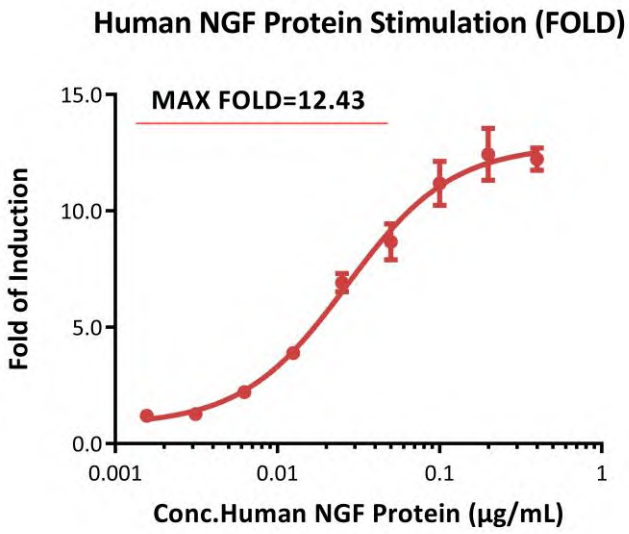

Fig3. Response to human NGF protein (FOLD). The Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat. No. BEF-H5214). The max induction fold was approximately 12.43.

- ***Application***

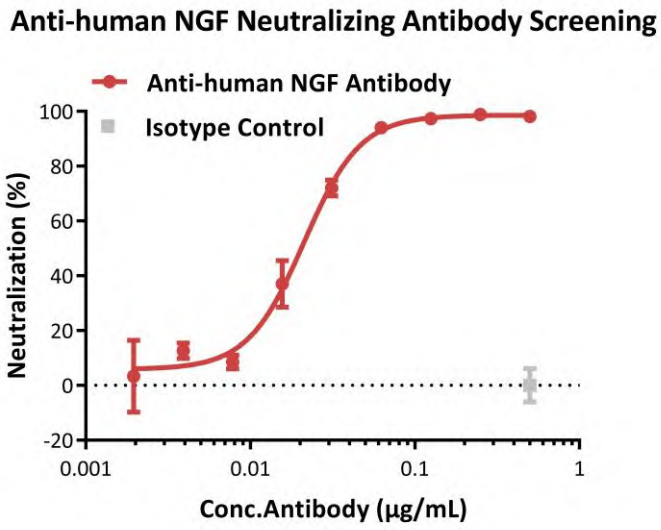

Fig4. Inhibition of human NGF protein-induced reporter activity by anti-human NGF neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human NGF protein (Cat. No. BEF-H5214) with a final concentration of 0.05 μg/mL. The EC50 of anti-human NGF neutralizing antibody is approximately 0.0212 μg/mL.

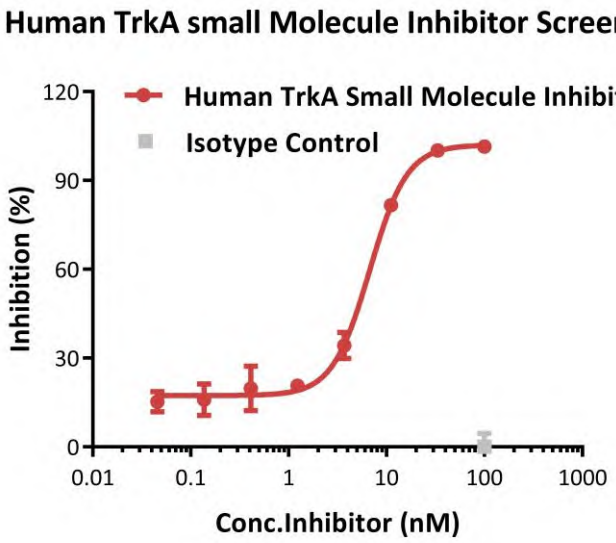

Fig5. Inhibition of human NGF protein-induced reporter activity by human TrkA small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human NGF protein (Cat. No. BEF-H5214) with a final concentration of 0.03 μg/mL. The EC50 of human TrkA small molecule inhibitor was approximately 6.725 nM.

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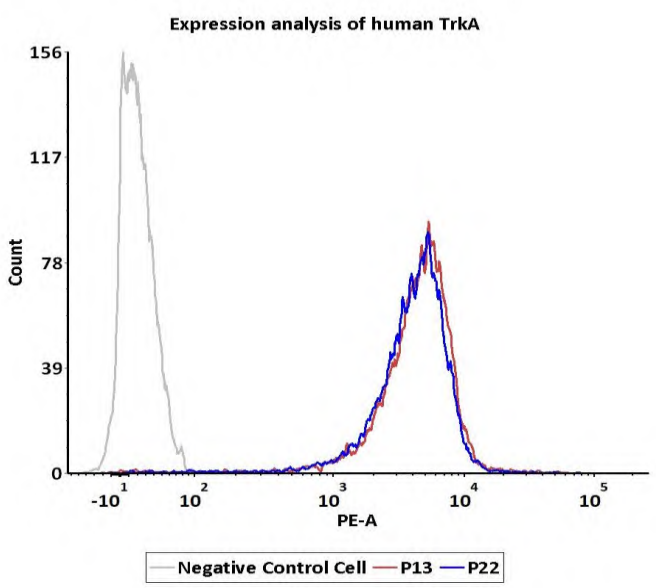
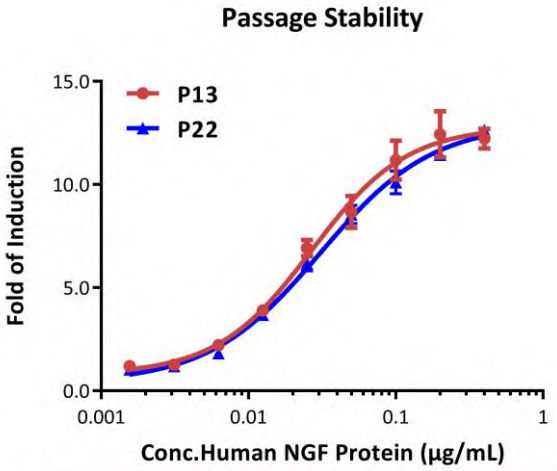
- ***Passage Stability***



Passage	MFI for TrkA (PE)
P13	4437.83
P22	4138.28

Fig6. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human TrkA on Human TrkA (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 13-22.



	P13	P22
EC50 (μg/mL)	0.02679	0.03079
Max Fold	12.43	12.63

Fig7. Passage stability analysis by human NGF protein stimulation. The continuously growing Human TrkA (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human NGF protein (Cat. No. BEF-H5214) stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 13-22.

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• *Related Products*

Products	Cat.No.
Human NGF protein	BEF-H5214
HEK293/Human APP (GFP) Stable Cell Line	CHEK-ATP081
HEK293/Human TrkB Stable Cell Line	CHEK-ATP082
HEK293/Human Alpha-synuclein (GFP) Stable Cell Line	CHEK-ATP085
HEK293/Human Tau-K18 (GFP) Stable Cell Line	CHEK-ATP087
Human 5-HT1A (Luc) HEK293 Reporter Cell	CHEK-ATF131
HEK293/Human SORT1 Stable Cell Line	CHEK-ATP155
HEK293/Human RAGE Stable Cell Line	CHEK-ATP156
HEK293/Human NGFR Stable Cell Line	CHEK-ATP157
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158
HEK293/Human LILRB3 Stable Cell Line	CHEK-ATP159
Human CGRPR/RAMP1(Luc) HEK293 Reporter Cell	CHEK-ATF168
HEK293/Human TrkC Stable Cell Line	CHEK-ATP189
HEK293/Human TrkA Stable Cell Line	CHEK-ATP192